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Reactions of carbonyl compounds with Amberlite IR-45

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REACTIONS OF CARBONYL COMPOUNDS WITH AMBERLITE IR-45

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CHAPTER I

INTRODUCTION

Organic chemistry, as the name implies, was originally the study of "organic" or naturally occurring compounds, as urea, acetic acid, alcohol, and alkaloidal drugs. As factual knowledge and theory of the field expanded, many classes of compounds of non-natural origin have become of interest. In fact, the greater weight of commercial interest has centered on the synthetics and a few natural raw materials e.g. sugar, cellulose, etc. Almost without exception, these substances were obtainable in the pure state in comparatively large quantities and their isolation presented few difficulties.

However, the chemical purist has long been fascinated with the why and how of the chemistry of life. In recent years, this interest has become more and more withdrawn from its academic seclusion by its development to a level of sophistication such that real commercial benefits, as the control of plant growth and of metabolic disorders, become feasible.

The very chemical complexity of the living system has focused interest on compounds of physiological importance such as vitamins, hormones, and mold products. That these substances are present in small amounts only furthers the

complexity of the mixtures, and the close familial relationships of many of the components mean that separations are often most difficult. In fact, it is no exaggeration to say that our knowledge in this field is often primarily bounded by the limitations of separative methods presently available. Classical methods used for separating substances from each other have been based upon single stage operations such as crystallization, distillation, extraction by different solvents, and precipitation. These are often clumsy, wasteful, and time consuming.

In chromatography, we have a preparative and analytical method based on the counter-current principle. The impact of chromatography upon chemistry can best be realized by considering the tremendous explosion of knowledge in the field of carbohydrate and protein chemistry occasioned by the development of paper partition chromatography by Martin and Synge in 1941.

Partition chromatography is a method of separating substances by distributing them between two liquid phases, one of which is mobile and the other essentially fixed by sorption to a support. The success of partition chromatography is due largely to the fact that it represents the first practical application of counter-current fractionation procedures (classically exemplified by fractional distillation) to substances of the lability characteristic of natural

products. It is unnecessary to delineate the advantages offered by counter-current procedures compared to "one shot" separations such as distillation, crystallization, and partition by separatory funnel.

It has been reported by several authors that reducing sugars are undesirably retained by strong anion exchange resins during attempted removal of sugar acids by the resin (Hulme, 1953, Phillips, 1953). This retention of reducing sugars on the hydroxide form of resins was regarded as an undesirable reaction, which led to complications in the interpretation of analytical results. It was proposed by Dr. W. H. Wadman that this retention might be utilized as an analytical tool; namely, that aldehydes and ketones would undergo sorption with amine exchange resins and could be removed from dilute solutions in which they were impurities.

The chromatographic process involves the distribution of a solute, sometimes called the "adsorptive", between two phases, one of which is stationary, or immobile, and the other, mobile. Speaking in a relative sense, this represents a counter-current distribution. In paper chromatography, the immobile phase is considered to be the water molecules bound into the cellulose network of the paper, whereas the mobile phase may be any one of a number of pure or mixed solvents. Distribution of the solute between the "bound" water and the mobile phase results in movement of

the solute through the paper. In adsorption chromatography, the immobile phase is a solid adsorbent, and the mobile phase may be one of several pure or mixed solvents. The process of adsorption in chromatography is often considered as a competition between the adsorptive and the developing solvent for sites on the adsorbent surface. Thus, if the attraction between the developing solvent and the adsorbent is higher than that of the adsorptive for the adsorbent, the adsorptive will find itself moving along with the solvent. If the reverse occurs, the adsorptive will tend to stick to the column and thus be retained. Dielectric constants, dipole moments, hydrogen-bonding ability, and relative polarizabilities of the three components are among the factors that decide the outcome of this competition. The separation of stereoisomers implies that the shape of the molecule plays an important role in chromatographic analysis (Zechmeister, 1948).

It is the purpose of this thesis to explore the experimental relationships which exist between Amberlite IR-45 and various carbonyl compounds with the hope that the knowledge obtained may ultimately form the basis of a new separative technique.

CHAPTER II

HISTORY OF CHROMATOGRAPHIC METHODS

Although the Russian botanist, M. Tswett, has been credited with the discovery of the principle of passing a solution through an adsorbing column to separate it into two or more distinct compounds (1906), records as far back as the time of Aristotle indicate that sand filters were used for the purification of drinking waters. The first paper published by Tswett contained a study of more than one hundred adsorbents used in conjunction with several different solvents and a comparison of the efficiency of column and batch adsorption. Many naturally occurring materials as silk, wool, cellulose, and cell membranes exhibit the phenomenon of selective adsorption and retention.

Over one hundred years ago, the agricultural chemists, Thompson and Way, began their classic work on ion exchange with clays. Since that time, tremendous advances have been realized in the theory, practice, and commercial development of ion exchangers. By 1900, ion exchange processes were utilized to convert calcium salts in beet sugar juice to potassium salts with the object of increasing the yield of crystallizable sugar, as a water softening agent, and to separate pigments of biological origin. In the early days

of chromatography, the difference between adsorption chromatography and ion exchange chromatography was not clearly recognized.

Within the past twenty years, many new synthetic organic ion exchangers have been prepared but only utilized to a small percentage of their potential. The use of ion exchange resins as adsorption and partition chromatography agents was begun about 1935 when Adams and Holmes snythesized the first synthetic organic exchanger. Until that time, only naturally occurring exchangers were utilized; all except the mineral apatite were cation exchangers. The discoveries of Adams and Holmes opened a new era in the manufacture and use of substances with exchange properties. About the same time, similar efforts appear to have been initiated at the I. G. Farben Laboratories. A number of interesting exchangers were produced and some of their properties have been described by Griessbach (1939).

A second and highly significant step in the evolution of technology of the production of ion exchangers took place in connection with the vinyl polymer field. The initiation of this important new basis for preparing organic ion exchangers was due to D'Alelio (1942). He based his invention on the idea of building up an inert, three dimensional, crosslinked hydrocarbon network structure by the co-polymerization of styrene with divinylbenzene to which

ionogenic groups could be attached to confer the ion exchanging property upon the resulting material.

Proceeding parallel with the development of the technology of the production of ion exchange resins has been the simultaneous advance in the theory and utilization of chromatographic systems. During the years following Tswett's classic work on chlorophyl pigments, his method was used only rarely. Workers such as Palmer and Eckles (1914), and Kuhn and Lederer (1931), made use of Tswett's chromatographic procedures, although only minute quantities of pigments were recovered from their columns.

In order to effect the separation of a mixture of amino acids, Martin and Synge (1941) utilized the difference of partition coefficients with a battery of solvent-solvent extractors. Although this work with counter-current extractors has been further developed by Craig (1950), Martin and Synge found that a far more efficient fractional solvent-solvent extraction was possible by packing columns with silica gel holding about 50% water, placing the solution of a mixture on the column, and developing with suitable solvents. Other materials also capable of holding water as a stationary phase are cellulose, starch, Kieselguhr, and glass powder. This procedure was named "partition chromatography" and was the classical work that gave chromatography a tremendous impetus.

In 1944, Consden, Gordon, and Martin showed that filter paper sheets and strips can be utilized to support a stationary phase. Their technique, called "paper chromatography", is probably the most versatile means devised for the resolution of micro quantities of chemicals.

Work on the theory of chromatography commenced about 1940; prominent contributions have been made by Wilson (1940), Martin and Synge (1941), DeVault (1943), Glueckauf (1945), Holm (1954), and Gregor et. al. (1956).

A sampling of the current literature shows the following widely varied uses of synthetic organic resins in organic chemistry: refining of beet sugar, purification of ethylene glycol, removal of acidic and basic properties, recovery of malic acid from apple syrup, separation of carbohydrates, separation of peptides and amino acids, catalysts in hydrolysis of esters and carbohydrates, separation of fission products and rare earth metals, purification of various types of viruses, preparation of cytochrome, and isolation and purification of antibiotics. Now that scientists have become aware of the wide applicability, the extreme sensitivity, and the great rapidity of chromatographic methods, hundreds of new uses and numerous modifications have been listed.

Several workers have studied some aspects of the relationship between carbonyl compounds and ion exchangers.

Thus, Samuelson (1950) used a bisulphite resin to remove aldehydes and ketones from alcohol solutions. Sugars form bisulphite compounds, but these are so unstable in water solution that the binding of sugars by bisulphite resins is inappreciable. In 1954, Yoshiro studied the adsorption equilibrium between formaldehyde and resin exchangers containing primary and secondary amines (among which was Amberlite IR-45). He found that a condensation reaction occurred between the resin and formaldehyde and that aromatic aldehydes are adsorbed by resins containing primary amino groups.

The efficiency and convenience of chromatographic separations, in relation to those obtainable by other methods, vary greatly with the field of investigation. Applied to some substances, such as sugars, each new application of the chromotographic technique has revealed many new compounds that have escaped detection or isolation by other methods.

CHAPTER III

THEORY

I. RESIN ALDEHYDE REACTION

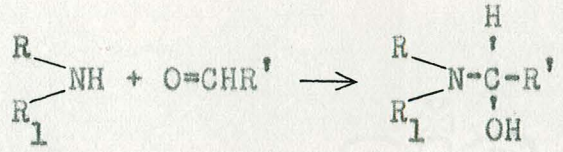
It is germane to our study to consider the classical reactions of monomeric carbonyl compounds and amino compounds as a basis of reference for the attempted interpretation of the presumable analogous reactions occurring by adsorption of carbonyl compounds on amino ion exchange resins. It is known that aldehydes and ketones will condense with primary and secondary amines to form a nitrogen containing derivative but do not react with tertiary amines.

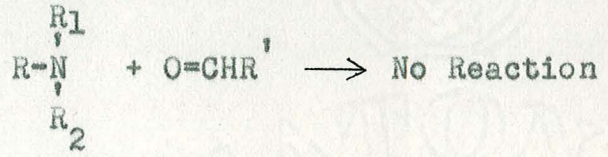
How than may an amino resin containing a mixture of primary, secondary, and tertiary amino groups react to remove aldehydes and ketones from solution?

The amine resin is first regenerated from the chloride form to the amine form. As aldehydes and ketones are mixed with the resin, the following reactions will occur. (Illustrated by an aldehyde.)

1. $RNH_2 + O{=}CHR' \longrightarrow R{-}N{=}CHR' + H_2O$

2. $RNH_2 + O{=}CHR' \longrightarrow R{-}NH{-}\underset{\displaystyle OH}{\overset{\displaystyle H}{C}}{-}R'$

3. 

4. 

Thus, both the mixture of reaction 1, 2, and 3 possibly occurring simultaneously and the irregular structure of the resin polymer inevitably results in sites of differing absorbtive capacities so that the kinetics of absorption can hardly be expected to be as simple as the foregoing equations suggest.

II. RESIN STRUCTURE

The resin used in these experiments was the weakly basic anion exchange resin, Amberlite IR-45, analytical grade, 16-50 mesh, a product of Rohm and Haas Company. This resin may be regarded as a high molecular weight insoluble base with primary, secondary, and tertiary amino groups as the active groups. Two important requirements of an exchanger are insolubility and chemical stability. These can be realized only if the active groups are attached to a crosslinked skeleton. Although extensive patent literature has come into existence dealing with the technology of the production of ion exchange resins, few scientific publications have appeared in which sufficiently clear

details have been given as to their probable chemical composition and structure. A new basis for preparing organic ion exchangers was proposed by D'Alelio (1942). He based his invention on the idea of building up an inert, three dimensional, cross-linked hydrocarbon network structure by the co-polymerization of styrene with divinylbenzene to which groups could be attached to confer the ion exchange property upon the resulting material. The chemical structure for the polymer is a linear polystyrene chain cross-linked by divinylbenzene. The extent of cross-linking may be varied over wide ranges by changing the proportion of divinylbenzene. This product is the starting point for the manufacture of a number of weakly basic amino anion exchangers. It is believed that there is but one anion exchange group per every two benzene nuclei. An organic ion-exchange resin may be described as a cross-linked, three dimensional molecular network containing structurally bound ionogenic groups. The number of these groups per unit mass determines the ion exchange capacity, usually expressed as meq. per gram. Capacities averaging 9.5 to 10.0 meq. per gram of dry resin have been reported. (Lederer, 1953)

When dry resin is immersed in distilled water, water is taken up by the polar groups until they become fully hydrated, after which the absorption becomes more gradual. If dry resin is immersed in an aqueous solution of maltose,

starch, etc., only water of solution is absorbed by the resin, thus concentrating the solution (Wadman 1952). For this reason, all the work with Amberlite IR-45 is done with wet resin.

Wet resin may be considered to contain water of two types: non-solvent water associated with structurally-bound exchange groups and solvent water which is held on the surface, in cracks and crevices, of the resin. The exchanging ions are considered to be dissolved in solvent water only and are able to escape into external solutions freely. Thus, the manufacturer recommends that the resin be kept wet at all times. The moisture content of the drained resin was almost 46%. The work of Webber showed a lower percentage of water in the resin, but the difference in these figures is accounted for by the techniques applied in preparing the resin for use. Due to the fact that the water content varies with the particular method of preparing the resin, it seems best to quote resin weights in all experimental data on the basis of dry resin. A further problem arises from the necessary use of wet resin. The greater portion of the water introduced with the resin is solvent water and, hence, serves to dilute any solution to which the resin is added, introducing a spurious apparent absorption. Thus, all supernatant concentration figures must be appropriately adjusted, e.g. in 1.5 g. of wet resin there is

0.69 g. of water which mixes with the original 50 ml. of solution used in the resin carbonyl experiments. This 0.69 g. gives a new volume of 50.69 ml. and the concentration of supernatant must be increased by $\frac{50.69}{50}$ to eliminate error from this dilution.

Since anion exchangers are regarded as high molecular weight organic polybases, they show characteristic electrochemical properties. When titrated with strong acids, they give pH titration curves resembling curves obtained by the titration of strong acids and weak bases. Ion exchangers exhibit ionic conductivity and ionic transport phenomena, which requires that in cases where potentiometric titrations are conducted on supernatant solutions, the resin must be removed by filtration before such a titration.

Mention should be made of certain experimental difficulties which may limit the accuracy of the experiment. Although, ideally, these high molecular weight polybases might be regarded as insoluble, when dry resin is put into water, it has been found to shed relatively low molecular weight soluble material which is either displaced or broken off from the network. These soluble fragments will sequester ions and may give misleading experimental values for the concentration in the external phase.

It was proposed by Webber (1958) that steric hindrance may block many potential resin-aldehyde reactions. If the

amine groups on or near the surface of the resin react with aldehyde groups, the diffusion of more aldehyde to the inner portion of the resin beads may be blocked in such a manner that relatively few of these active groups are able to penetrate. We might add to this theory the possible hindrance offered by areas of dense cross-linking within the resin molecule.

Since commercial resins represent a compromise in which the cross-linking is not great enough to impede seriously the diffusion of ions but is sufficient to prevent large swelling effects, we shall consider it to be an adsorbent with a very large internal surface. The added solutes are considered to be organic nonelectrolytes, so interfacial effects cannot be ignored and preferential adsorption on the resin network becomes important. In equilibria involving an aqueous solution of two organic components and the resin, the affinity of the resin for both compounds will differ, depending upon K values for each reaction, polar affinity degree of adsorption, and molecular size.

It is now apparent that the rate of approach to equilibrium of organic nonelectrolytes upon ion exchange resins depends upon: (a) concentration of the solution, (b) degree of stirring, (c) particle size of resin, (d) diffusion coefficients of the organic compounds, (e) steric

hindrance, (f) degree of cross-linking, and, (g) temperature. In practice, all these factors are found to influence the rate of sorption.

III. CALCULATION OF THE AK VALUE

If the reaction may be expressed by the simple equation

$$5.\quad -NH_2 + O{=}CHR \longrightarrow -N{=}CH{-}R$$

the equation at equilibrium may be stated as

$$\underset{\text{Resin}}{A} + \underset{\text{Aldehyde}}{B} \longrightarrow \underset{\text{Resin-Aldehyde Complex}}{C} \qquad 6.$$

with A, B, and C representing the concentrations of the unused resin, unreacted aldehyde, and resin-aldehyde complex respectively. The value of the equilibrium constant may be found by the classical law of mass action where

$$7.\quad \frac{C}{AB} = K$$

By direct analytical methods, the value of B may be obtained; thence, the value of C is computed by difference, but the value of A still remains unknown. The value of A must be obtained before the value of K can be calculated.

Let

$$8.\quad A + C = D$$

where D is defined as the limit capacity of the resin. In equation 7, as A approaches zero, B approaches infinity or $\frac{1}{B}$ approaches zero. In equation 8, as A approaches zero, C approaches D. A plot of $\frac{1}{B}$ vs. C gives a curve which may be extrapolated for the value of D. Once the value of D is known, the value of A may be calculated from equation 8 and the value of K computed (Webber).

This discussion has presupposed that in the simple expression of the law of mass action, more than one molecule of resin or aldehyde was not consumed. If this is so, then a plot of $\frac{C}{B}$ vs. C is linear for all concentrations of B. This has been investigated by Webber who decided that the simple plot did not fit the data obtained perfectly but was the best. We are, of course, more interested in the linearity of $\frac{C}{B}$ vs. C at very low concentrations of aldehyde such as are envisaged for an actual separative technique, so error in evaluating D and hence K is not serious for our purposes. In fact, it should be clearly recognized that the failure to obtain a linear plot is to be expected since, as has been explained, the reaction must inevitably be far more complex than that treated above.

Another factor which should be mentioned is that Amberlite IR-45 is composed of 60% tertiary amines and 40% primary and secondary amines mixed. Each primary and secondary amino group has a different K value for the

equilibrium established between aldehyde and resin.

A method for obtaining the relative values of K for different aldehydes can be obtained from equation 7 by multiplying both sides of the equation by A

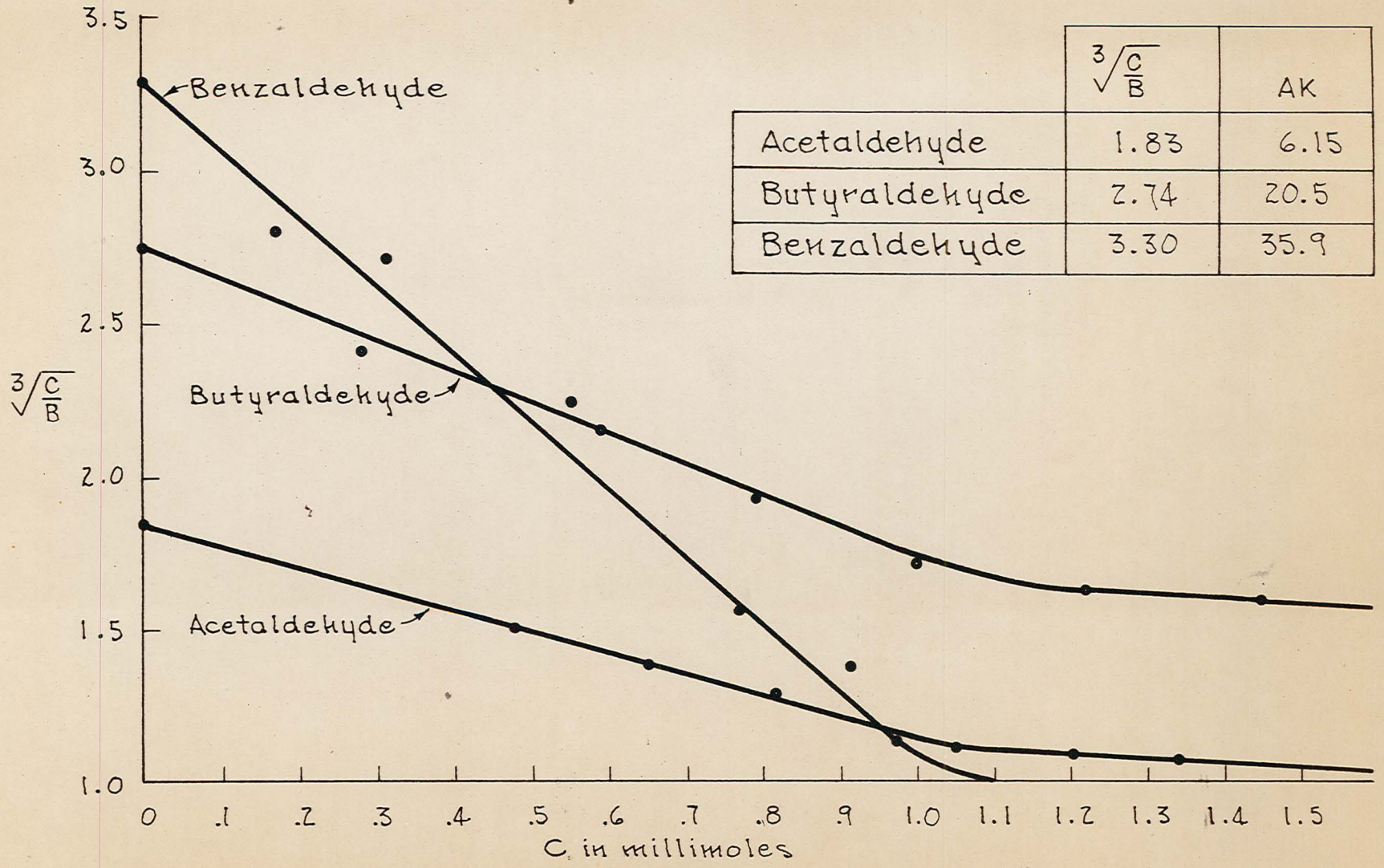
$$9. \quad \frac{C}{B} = AK$$

The plot of $\frac{C}{B}$ vs. C, when extrapolated to C=0, gives the value of AK. As C approaches 0, the amount of unreacted resin is small and since, therefore, A tends to become constant, we can obtain the relative values of AK for the different aldehydes. Since extrapolation of a linear plot is preferable, we find more precision is obtained by plotting $\sqrt[3]{\frac{C}{B}}$ rather than $\frac{C}{B}$ itself. Extrapolated values of AK were found to be as follows: acetaldehyde 6.15, butyraldehyde 20.5, and benzaldehyde 35.9. (Figure 1)

While the Ak values stand rigid in relation to one another and since the absolute value of A cannot be readily determined, then only relative values of K can be determined.

From the work of Webber we find the value of A to be 5 meq. per gram of dry resin. The value of K for acetaldehyde is then calculated as 1.23, butyraldehyde is 4.10, and benzaldehyde is 7.17.

We prefer to quote AK values instead of K values. Webber has clearly demonstrated the difficulty of assigning a rigid value for A and for chromatographic purposes there



	$\sqrt[3]{\frac{C}{B}}$	AK
Acetaldehyde	1.83	6.15
Butyraldehyde	2.74	20.5
Benzaldehyde	3.30	35.9

FIGURE 1

EXTRAPOLATED VALUES OF AK

is no great value for getting K separate from AK. In fact, for chromatographic purposes, since AK = $\frac{C}{B}$ i.e. concentration of adsorbed solute divided by concentration of dissolved solute, an AK value may be regarded as a form of the partition coefficient and, hence, has greater utility than K alone. To achieve separation of compounds on a resin column, it is only necessary that the AK values be different. The greater is the difference, the more ready will be the separation.

The large difference between the AK values of acetaldehyde and benzaldehyde indicates that there should be a separation on a column of Amberlite IR-45. This experiment was performed and, by frontal analysis, acetaldehyde was found to emerge from the resin column before the benzaldehyde.

IV. ANALYTICAL METHODS

The carbonyl analytical procedure developed by Webber, total oxidation of aldehyde to the corresponding acid by acidic dichromate followed by titration, was, at its best, a relatively crude method. The myriads of oxidation products obtained could cause serious experimental error. It was found that his method would not work in the presence of the amino resin and that the precision of the work was not good when working with low concentrations of aldehyde.

The necessary features of an "ideal" analytical technique would permit direct carbonyl analysis in the presence of the amino resin, consume little time, possess a very high degree of accuracy, employ solutions that would not change titer on standing for periods of time, and be generally applicable to water soluble carbonyl compounds.

A survey of the existing analytical methods was undertaken to determine if one could be utilized in this research. A great number of procedures are available for the determination of aldehydes and ketones, most being very specific for the more active compounds. Thus, several methods for determining aldehydes are based upon the ease with which the aldehydes are oxidized. Although most of the early analytical developments were directed toward the determination of specific compounds, such as formaldehyde and acetone, later investigations often demonstrated that the procedures had much wider applicability.

Most of the general methods are volumetric based on reaction followed by titration of a reaction product or of excess reagent. Volumetric methods are usually applicable to a wide concentration of free carbonyl groups. These relatively simple techniques are most accurate for the analysis of mixtures containing appreciable amounts of aldehyde or ketone. A few gravimetric procedures have general applicability. Usually, gravimetric methods are

most useful for determining certain carbonyl compounds in complex mixtures. Colorimetric methods are useful for determining small concentrations of carbonyl compounds. Sensitive techniques are available and employ instruments for the final measurement. Instruments, such as the infrared spectrometer and the polarograph, are finding increasing use for the direct determination of specific aldehydes or ketones. Carbonyl group absorptions in the ultraviolet region of the spectrum can often be employed in quantitative analysis.

The analytical procedures investigated are as follows:

Hydroxylamine Hydrochloride--Pyridine Procedure

The reaction of carbonyl and hydroxylamine hydrochloride forms the basis of the most widely applicable chemical technique for determining the carbonyl function. The general applicability of oximation to the determination of aldehydes and ketones has been shown by several investigators (Bennett, Bryant and Smith, and Schultes).

$$RCHO + H_2NOH \cdot HCl \longrightarrow RCH{=}NOH + HCl + H_2O$$

$$RCOR' + H_2NOH \cdot HCl \longrightarrow RR'C{=}NOH + HCl + H_2O$$

Most of the reported analysis are based on titration of the released acid with standard alkali. These titrations

may be visual or potentiometric. Two reagents were used by Bryant and Smith, i.e. 0.5N hydroxylamine hydrochloride in 80% ethanol and 2% pyridine in 95% ethanol containing bromphenol blue indicator. Pyridine is used to aid in forcing the oxime formation to completion, to act as an acid acceptor, and to render the initial reagent neutral to bromphenol blue. Active aldehydes and ketones react completely at room temperature while the more stable carbonyl compounds require one or more hours at 98°--100° C. Hydroxylamine hydrochloride must be standardized daily.

Colorimetric 2,4-Dinitrophenylhydrazine

The most widely applicable colorimetric procedures are based on the use of 2,4-dinitrophenylhydrazine. In 1920, Mathewson described a method for the estimation of acetone. Colors developed in alkaline solutions of the 2,4-dinitrophenylhydrazones were used in determining pyruvaldehyde (Barrenscheed and Dreguss) and furfural (Barta). Lappin and Clark demonstrated the general applicability of this technique for low concentrations of carbonyl compounds in solutions.

$$RCHO + H_2NNH\cdot C_6H_3(NO_3)_2 \longrightarrow RCH{=}NNH\cdot C_6H_3(NO_3)_2 + H_2O$$

Colorimetric Iodoform Procedure

One of the oldest and best known qualitative tests

for organic compounds containing the aceto group, or a group capable of easy oxidation by alkaline hypiodite to the aceto group, is the iodoform reaction:

$$R{\cdot}CO{\cdot}CH_3 + 4NaOH + 3I_2 \longrightarrow CH_3I + 3NaI + RCOONa + 3H_2O$$

Iodoform absorbs in the ultraviolet region from 400 to 260 mu. Three well defined maxima occur at 347, 307, and 274 mu. The absorption peak at 347 is the most sensitive to changes in iodoform concentration (Dal Nogare et.al.). Ultraviolet absorption by iodoform thus gives a sensitive and accurate means for determining acetaldehyde and acetone in low concentrations.

Sulphite--Acid Procedure for Aldehydes

The fact that aldehydes add bisulphite has been known for many years. Ripper's method, in which aldehydes react with bisulfite and the excess bisulfite is determined by iodine oxidation, has undergone much modification. Because of the instability of bisulfite solutions, Siggia and Maxey found it advantageous to use sulfuric acid instead. An aliquot of standard sulfuric acid is added to a large excess of sodium sulfite solution so that sodium bisulfite is essentially the active ingredient.

$$2Na_2SO_3 + H_2SO_4 \longrightarrow Na_2SO_4 + 2NaHSO_3$$

$$NaHSO_3 + R\text{-}CHO \longrightarrow RCHOH\text{-}SO_3Na$$

The acid is stable and can stand long periods without changing titer. It is added to the sulfite just before the aldehyde sample is introduced. The aldehyde reacts rapidly with the bisulfite, and the excess bisulfite is titrated with standard alkali.

The large excess of sulfite is to keep the reaction at completion as the excess bisulfite is titrated with alkali. The reaction is so near completion that no odor of aldehyde can be detected above the solution.

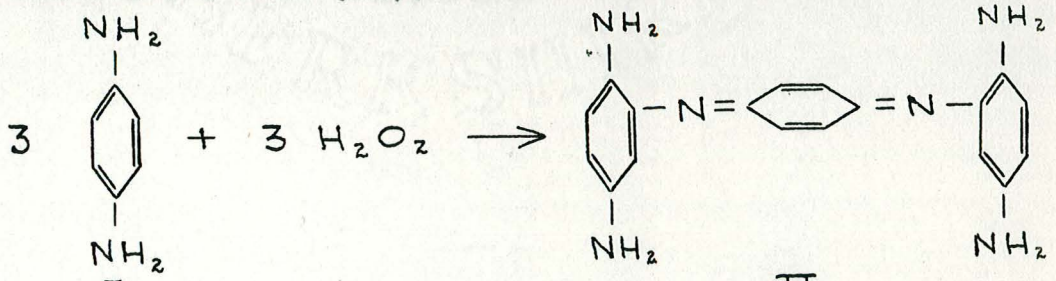
To detect the endpoint, a pH meter is used in the titration. A curve is plotted of the pH of the solution versus the milliliters of standard alkali added, and the endpoint is determined from the curve. The endpoint in each case comes at a definite pH. Once this pH is known, each sample can be titrated to it, eliminating the necessity of plotting a curve for each sample. Precision is ±0.2% when the curve is plotted and ±0.4% by the more rapid method.

Spot Tests

Spot tests were utilized to identify acetaldehyde and benzaldehyde as they emerged from the resin column. Each test had to be specific for a carbonyl compound and also work in the presence of the other aldehyde.

In acid or neutral solution, p-phenylene diamine (I)

is oxidized by hydrogen peroxide to a black compound known as Bandowski's base (II).



I II

The velocity of this reaction is increased by aldehydes. In neutral solution, aldehydes produce a black color or precipitate, preceded by other transitor coloration. Aliphatic aldehydes react the same in both acid and neutral solution, but aromatic aldehydes form a yellow color which lasts for some time in acid solution.

Another spot test for acetaldehyde was also used. The aldehyde reacts with sodium nitroprusside and alkali to produce an intense yellow color. The mechanism of the reaction is not clear.

From the above series of analytical procedures, the following were selected as being the best suited for the experiments performed:

A. Colorimetric Iodoform--selected for acetone analysis because of its accuracy, use of stable solutions, and the reproducible curves acquired.

B. Sulfite--Acid Procedure--selected for analysis of all carbonyl compounds because of its rapidity, stable

solutions employed, high degree of accuracy, and simplicity.

CHAPTER IV

ANALYTICAL PROCEDURES

Carbonyl compounds, aldehydes and ketones, have in common the relatively reactive C=O group. Specific methods of analysis are available for certain of the more active materials. Several methods for determining aldehydes are based on the ease with which the aldehydes are oxidized to the corresponding acid. Webber used this fact as the basis of his analytical procedure.

Several analytical procedures were investigated in an effort to find one that was applicable to this research.

I. HYDROXYLAMINE HYDROCHLORIDE PYRIDINE PROCEDURE (BRYANT AND SMITH)

Precisely 30 ml. of 0.5N hydroxylamine hydrochloride in 80% ethanol and 100 ml. of pyridine reagent (20 ml. of pyridine plus 0.25 ml. of 4% alcoholic bromphenol blue per liter of 95% ethanol solution) are transferred to a pressure bottle (Erlenmeyer flask and stopper). The sample is added. The bottle is capped and allowed to stand at room temperature one hour. The hydrochloric acid released during oximation is determined by titration with standard 0.5N sodium hydroxide in 90% methanol until the color matches

that of a blank.

Results

The precision and accuracy of this method depends to a large extent on the ability of the analyst to match the endpoint with that of the blank. Accuracy is 1%. This method is affected by acidic and basic compounds and by large amounts of inert solvents; also, the hydroxylamine reagent is unstable, requiring standardization daily.

II. COLORIMETRIC 2,4-DINITROPHENYLHYDRAZINE (LAPPIN AND CLARK)

To one ml. of sample solution, adjusted to a concentration of 10^{-4} to 10^{-6}M, is added 1.0 ml. of 2,4-dinitrophenylhydrazine reagent (saturated carbonyl-free methanol solution) plus one drop of concentrate HCl. The mixture is heated on a water bath at 50° C. for 5 minutes. After cooling, 5.0 ml. of 10% potassium hydroxide in 80% aqueous methanol are added. The nearly black solution clears almost immediately to a wine-red color. The absorbancy is determined by reference to a standard curve.

Results

The solution was found to be too dark to distinguish between the blank and the unknown. The time to perform the complete analysis took 30-45 minutes.

III. COLORIMETRIC IODOFORM PROCEDURE (NOGARE, NORRIS, MITCHELL)

Ten ml. of 20% iodine solution are pipetted into a 125 ml. separatory funnel and 3.3 ml. of 20% sodium hydroxide are added. If the resulting solution is not distinctly orange-yellow, adjust it to this endpoint by adding iodine solution, one drop at a time. To this hypoiodite solution is added, from a pipet, 1-5 ml. of sample containing no more than 0.4 mg. of acetone or acetaldehyde and shaken immediately.

Stopper the funnel and allow to stand for five minutes at room temperature. If the orange-yellow color is gradually discharged during the five minute reaction time, add iodine solution one drop at a time until the color is restored.

After reaction, discharge the iodine color with a few drops of 5% sodium thiosulfate. The iodoform is extracted with 22-24 ml. of chloroform. After being washed with an equal volume of water, transfer the chloroform layer to a 25 ml. volumetric flask, passing it first through an eyedropper containing a layer of anydrous sodium sulfate supported on a glass wool plug. This is to remove droplets of water. Make the extract to volume by passing chloroform through the sodium sulfate to wash down retained extract.

Measure the absorbancy at 347 mu. of the chloroform

extract against a chloroform blank in 2.5 cm. corex cells.

Results

By this analytical procedure, a sample could be analyzed for acetone content in 20-25 minutes. A standard curve was prepared (Figure 2) and results were found to be reproducible.

If too large quantities of acetone are used, a visible precipitate results. Because of the slow decomposition of iodoform in solution, optical measurements should be made as soon as possible. The critical study made by Hatcher and Mueller (1929) of the iodoform procedures for acetone showed that both the concentrations and the order of addition of reagents determined the yield of iodofrom obtained.

IV. SULPHITE--ACID PROCEDURE OF ALDEHYDES (SIGGIA AND MAXEY)

The analytical procedure used in this research is based upon the method of Siggia and Maxey but incorporates several changes in concentrations and volumes.

Fifty ml. of 0.05M sulfuric acid is added to 100 ml. of 0.125M sodium sulfite in a 250 ml. glass stoppered Erlenmeyer flask. To this solution is added a sample of aldehyde containing no more than 2.5 meq. The flask is

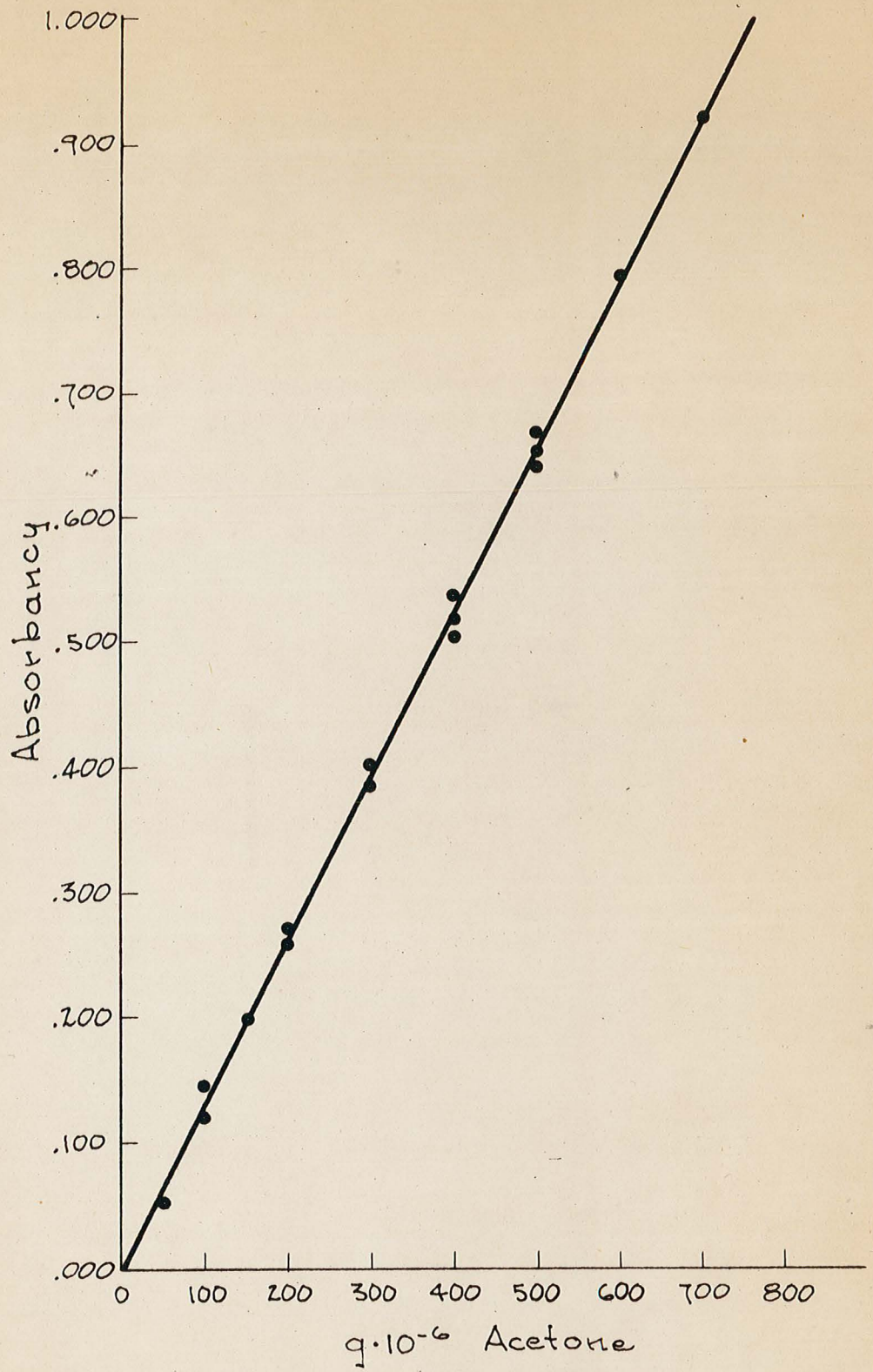


FIGURE 2

ABSORBANCY vs CONCENTRATION OF ACETONE

stoppered and shaken for at least three minutes to insure complete reaction. The contents are then transferred quantitatively to a 500-800 ml. beaker. Electrodes of a pH meter are inserted and the solution is stirred by a magnetic stirrer. Standard sodium hydroxide solution (0.1M) is added and the pH vs. ml. of alkali added is plotted. For the rapid determination, alkali is added until the endpoint pH is reached.

Results

With practice, easily reproducible curves can be obtained (Figure 3). Well defined endpoints were obtained using acetaldehyde, butyraldehyde, benzaldehyde, and cyclohexanone, but acetone gave a curve with no break. The procedure is accurate and simple to operate and was very applicable to the research.

V. SPOT TESTS

Two spot tests were utilized to detect acetaldehyde and benzaldehyde.

Spot Test A

A drop of the reagent solution (2% p-phenylenediamine), two drops of 2N acetic acid, and two drops of 3% hydrogen peroxide are mixed with a drop of the test solution on a spot plate. It is advisable to carry out a

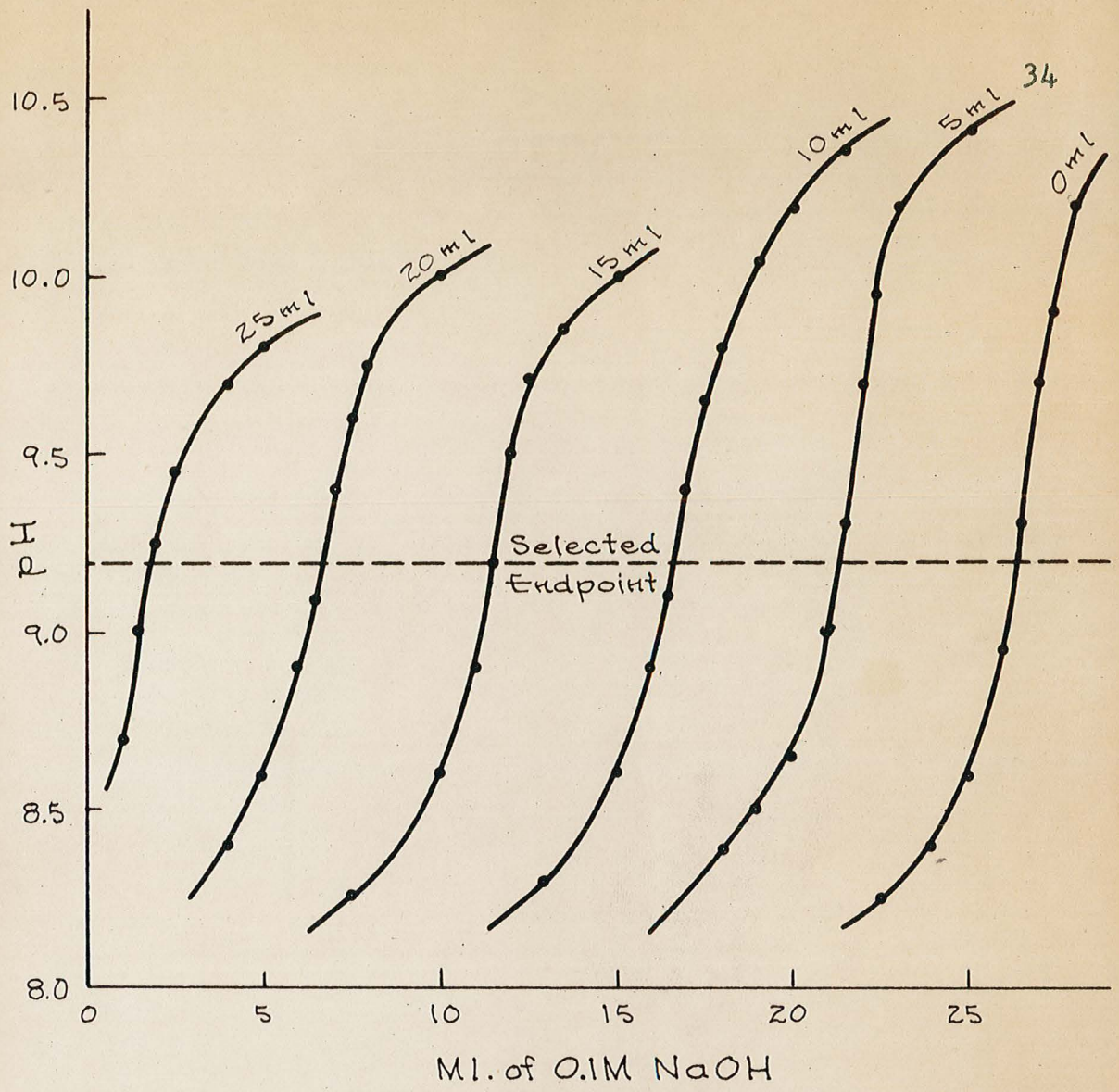


FIGURE 3

COMPOSITE GRAPH OF POTENTIOMETRIC CURVES FOR QUANTITIES OF 0.1M ACETALDEHYDE

blank test on a drop of water and also a parallel test omitting the acetic acid.

Results. Acetaldehyde gives a red spot to neutral and a green spot to acid p-phenylenediamine. Benzaldehyde gives a brown spot to neutral and yellow spot to acid p-phenylenediamine.

Spot Test B

A drop of test solution is mixed on a spot plate with a drop of 5% sodium nitroprusside solution and a drop of 30% sodium hydroxide.

Results. A definite orange color results from the above test on acetaldehyde. Benzaldehyde causes no color change. The acetaldehyde spot test works in the presence of benzaldehyde.

Summary of Spot Tests

The preceding spot tests can best be summarized as follows:

Compound	p-Phenylenediamine neutral	p-Phenylenediamine acid	Nitroprusside
Acetaldehyde	red	green	orange
Benzaldehyde	brown	yellow	no reaction
Mixture of two	black	yellow	orange

CHAPTER V

EXPERIMENTAL DATA

I. PREPARATION OF RESIN

Amberlite IR-45 resin was received from the manufacturer in the damp state and was transferred to a glass storage tube. This tube was thirty inches long and two inches in diameter, and the bottom was fitted with a rubber stopper and a 6 mm. length of glass tubing curved along the side of the storage tube to within four inches of the top. The 6 mm. tubing ended in a small siphon. The arrangement kept the resin under water at all times but permitted back-washing and other preparation to the column of resin.

The resin in the storage column was conditioned as follows: two liters of 1.0M HCl were percolated through the column followed by two liters of 1.0M NH_4OH. A very thorough back-washing with distilled water left the resin ready for future use.

When needed, about ten grams of resin were poured into a Buchner funnel and a slight vacuum applied for 15-20 seconds. This removed excess water but did not allow too much contact between the damp resin and CO_2 in the air. The required amount of resin was then weighed and placed in 50 ml. rubber stoppered Erlenmeyer reaction flasks.

II. RESIN ACETONE EXPERIMENTS

An experiment to study the reaction between acetone and Amberlite IR-45 was attempted.

Procedure

A 25 ml. aliquot of 10^{-3} g/ml. acetone solution was added to 0.09 g. of wet resin. After stoppering, the flasks were agitated for varying lengths of time (3-24 hours). The resin was removed by filtration and the liquid analyzed for acetone by the iodoform procedure.

Results. The percentage of acetone removed by the resin varied from 0% to as high as 29% on the same sample. It was believed the analytical technique was at fault.

Procedure

The experiment was repeated using 0.26 g. of wet resin (.432 meq.) and 25 ml. of 10^{-3} g/ml. acetone (.432 meq.) solution. The length of agitation ran from one hour to twenty hours. The acetone was analyzed by the iodoform procedure.

Results. Table I shows that acetone is not readily removed from solution by Amberlite IR-45.

Procedure

A third experiment used 25 ml. of 10^{-3} g/ml. (.432 meq.)

acetone and 0.26 g. (0.432 meq.) of resin. The acetone solutions were sealed in glass tubes and heated in boiling water for two hours. The tubes were cooled to room temperature before analysis by the iodoform procedure.

Results. Two tubes when analyzed showed no acetone had been removed. Another tube analyzed about one month later by a different analytical procedure yielded the same results.

Conclusion

This series of experiments revealed that acetone in dilute solutions (10^{-3} g/ml.) does not react with Amberlite IR-45 to any significant degree.

TABLE I

DATA FOR RESIN-ACETONE EXPERIMENT

Sample No.	Hours Agitated	$g \cdot 10^{-4}$ Acetone Added	Acetone Found	% Acetone Removed
1	1	25.0	23.8	4.8
2	2	25.0	24.3	2.8
3	4	25.0	25.0	0
4	8	25.0	25.0	0
5	20	25.0	24.8	0.8
Blank 1*	20.5	25.0	24.8	0.8
Blank 2**	21	0	0	0

*Blank 1--25 ml. of 10^{-3} g/ml. acetone, no resin

**Blank 2--25 ml. H_2O + 0.26 g. resin

III. ALDEHYDE RECOVERY EXPERIMENTS

Webber showed aldehydes could be removed from dilute solutions with Amberlite IR-45. His analytical procedure could not measure directly the amount of aldehyde absorbed by the resin.

The next experiments led to a technique that will allow the analyst to determine directly the aldehyde in the aldehyde-resin complex.

Procedure

Prepare six 50 ml. flasks as follows:

A. Add 25 ml. of H_2O.

B.,C. Add 5 ml. of H_2O and 20 ml. of 0.1M acetaldehyde.

D. Add 1.2 g. of resin and 25 ml. of H_2O.

E.,F. Add 20 ml. of 0.1M acetaldehyde, 5 ml. of H_2O and 1.2 g. of resin.

Each flask is stoppered and agitated for eight hours. Flasks A and B are control blanks.

Flasks C, D, and E were acidified with 25 ml. of 1.0M HCl to hydrolyze the aldehyde-resin complex. The contents of these flasks were then filtered, washed with 10 ml. of H_2O, and neutralized to pH 7 by the addition of 1.0M NaOH. The resultant solution was analyzed for aldehyde

by the sulfite-acid procedure.

The contents of flask F were separated by filtration. The filtrate was analyzed for aldehyde content while the residue (resin + resin-aldehyde) was acidified with 25 ml. of 1.0M HCl, filtered, the filtrate neutralized to pH 7, and then analyzed for aldehyde content by the sulfite-acid procedure.

Results. The latter procedure gave results indicating the aldehyde could be hydrolyzed from the resin-aldehyde complex and quantitatively determined (Table II).

TABLE II

DATA ON ALDEHYDE RECOVERY EXPERIMENT

Flask	meq. CHO Added	meq. CHO Found	% Aldehyde Recovered	
A	0	0	0	
B	2	2	100	
C	2	1.4	70	
D	0	0	0	
E	2	1.7	85	
F filtrate	2	1.3	65	95%
F resin		.6	30	

IV. CARBONYL AK VALUES

Now that a suitable analytical procedure had been found and proven useful, a series of experiments to determine AK values were performed. The carbonyl compounds used were acetaldehyde, butyraldehyde, benzaldehyde, acetone, and cyclohexanone.

Acetaldehyde Procedure

To twelve 50 ml. flasks were added 1.5 g. wet resin, multiples of 5 ml. of 0.1M acetaldehyde (0 to 50 ml.), and water to bring the liquid volume to 50 ml. The flasks were stoppered and agitated for five hours. At this time, the contents of the flasks were filtered, the resin washed with 10 ml. of water, and the filtrate analyzed for aldehyde content by the sulfite-acid procedure. Five blanks, containing varying amounts of aldehyde plus water but no resin, were run simultaneously and analyzed by the same procedure. The titration endpoint was pH 9.2.

Results. By extrapolation (Figure 1), the value of $\sqrt[3]{\frac{C}{B}}$ is found to be 1.83 and the calculated value of AK is 6.15.

Butyraldehyde Procedure

The butyraldehyde procedure is the same as that for acetaldehyde. The concentration of the butyraldehyde

solution was 0.1M. The titration endpoint was pH 9.2.

Results. The extrapolated value of $\sqrt[3]{\frac{C}{B}}$ (Figure 1) is found to be 2.74 and the calculated value of AK is 20.5.

Benzaldehyde Procedure

The benzaldehyde procedure is the same as that of acetaldehyde. Six flasks were prepared using 10 ml. multiples of aldehyde. Due to the low solubility of benzaldehyde in water, a saturated solution at 30° C. was used. The titration endpoint was 9.2.

Results. The extrapolation of Figure 1 gives a value of 3.30 for $\sqrt[3]{\frac{C}{B}}$ and the calculated value of AK is 35.9.

Acetone Procedure

See acetaldehyde procedure. The concentration of the acetone solution was 0.1M. Titration endpoint was 9.2.

Results. Acetone was not sorbed by the resin. The experiment tends to confirm work performed earlier using the iodoform procedure for analysis.

Cyclohexanone Procedure

See acetaldehyde procedure. The concentration of the cyclohexanone solution was 0.1M. Titration endpoint was 9.2.

Results. No significant degree of sorption of cyclohexanone was detected.

V. EQUIVALENT WEIGHT OF AMBERLITE IR-45

Procedure

The equivalent weight of the resin was determined by adding 50.0 ml. of 0.05M sulfuric acid to 1.5 g. of wet resin (0.69 g. dry resin) and agitating for twenty hours. The resin was removed by filtration, washed with 20 ml. of water, and the acid solution titrated potentiometrically with 0.1M sodium hydroxide solution.

Results. The equivalent weight of the resin was found to be 179 or 5.6 meq. per gram of dry resin.

VI. FRONTAL SEPARATION OF A MIXTURE OF TWO ALDEHYDES ON A COLUMN OF AMBERLITE IR-45

A small column, about 15 cm. long, was constructed of 10 mm. glass tubing. This column was simply a miniature resin storage tube as described previously. The column was packed with a slurry containing 1.5 grams of wet resin. The resin in the column was supported by a glass wool plug. The excess water was allowed to overflow through the syphon, leaving only enough water to cover the resin surface. The surface was then covered with small pieces of glass wool.

Procedure

A mixture containing one meq. of benzaldehyde and

one meq. of acetaldehyde per 50 ml. of solution was allowed to drop into the column from a separatory funnel at the rate of about 5 ml. per hour. The liquid emerging from the column was collected for half-hour intervals and spot tests were run on the fractions collected.

Results. Table III very clearly shows that a mixture of two aldehydes with a different AK value will emerge from the resin column at different times, one being selectively retained.

TABLE III

FRONTAL ANALYSIS OF ACETALDEHYDE AND BENZALDEHYDE

Sample	Time in Hours	Acetaldehyde	Benzaldehyde
1	0	-	-
	½		
2		-	-
	1		
3		-	-
	1½		
4		-	-
	2		
5		-	-
	2½		
6		-	-
	3		
7		+ ?	-
	3½		
8		+	-
	4		
9		+	-
	4½		
10		+	-
	5		
11		+	-
	5½		
12		+	+ ?
	6		
13		+	+
	6½		
14		+	+
	7		

CHAPTER VI

CONCLUSIONS

The experimental work showed that dilute solutions of acetaldehyde, butyraldehyde, and benzaldehyde were each sorbed by Amberlite IR-45. It is believed by the author that a resin-aldehyde complex was formed. The resin-aldehyde complex can be hydrolyzed with an acid solution and the aldehyde quantitatively recovered. Acetone and cyclohexanone do not form a resin-ketone complex. The experimental data shows that trace amounts of aldehydes can be removed from ketone solutions by passing the solution through a column of Amberlite IR-45 resin. The small quantities of aldehyde retained can be eluted from the column by acid treatment.

Furthermore, the Amberlite IR-45 column can be utilized to achieve separation of different aldehydes. This research has shown the extrapolated AK values to be similar to chromatographic partition coefficients and therefore, of practical value in estimating the feasibility of a chromatographic separation by this method.

A chromatographic separation of acetaldehyde and benzaldehyde by frontal analysis was successfully performed.

CHAPTER VII

SUMMARY

The two ketones used in this experimental work, acetone and cyclohexanone, were not found to react with Amberlite IR-45.

Acetaldehyde, butyraldehyde, and benzaldehyde were each sorbed by Amberlite IR-45. The acetaldehyde-resin complex was hydrolyzed with 1.0M HCl and the acetaldehyde was quantitatively determined by the sulphite-acid procedure.

Investigation was conducted to find an analytical technique that would permit direct carbonyl analysis in the presence of the amino resin. Due to the ionic conductivity and ionic transport phenomena of the resin, the resin must be removed by filtration before potentiometric titration. It was found that the sulphite-acid procedure was highly advantageous since the titrations could be done by potentiometric means, solutions were stable for long periods of time, analysis consumed little time, and the procedure possessed a high degree of accuracy.

Carbonyl AK values were obtained for acetaldehyde (6.15), butyraldehyde (20.5), and benzaldehyde (35.9). Using the value for A as 5 meq. per gram, calculated values of K were obtained for the following aldehydes: (a) acetaldehyde 1.23, (b) butyraldehyde 4.10, and (c) benzaldehyde 7.17.

The AK values were utilized to predict the separation of two aldehydes on a column of Amberlite IR-45. This separation was verified by the frontal analysis of the effluent liquid emerging from a column of resin upon which had been introduced a mixture of acetaldehyde and benzaldehyde solutions.

The equivalent weight of the resin was found to be 179 or 5.6 meq. per gram of dry resin.

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